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Note on the Natural Occurrence in Farm-raised Chickens of Encysted Third Stage Larvae of *Physocephalus sexalatus*, a Spirurid Stomach Worm of Swine

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BACKGROUND OF THE INVESTIGATION

The spirurid stomach worm of swine, *Physocephalus sexalatus*, utilizes as intermediate hosts various species of insects of the order Coleoptera, the third stage (infective) larvae being encysted in the body cavity and tissues of these insects. When infected beetles are consumed by swine, the larvae excyst and develop to maturity in the stomach of the host animal. Seurat (1912, Compt. rend. Soc. de Biol., Par., 72: 174–176) apparently was the first to report that when infected beetles are eaten by animals other than swine—the natural definitive host—the larvae are capable of reencysting in the wall of the alimentary tract of the aberrant host. This phenomenon was observed also by Cram (1930, The Auk, 47: 380–384) who fed infected beetles to pigs and a number of other animals, including the screech owl, turkey, quail, chicken, pigeon, skunk, opossum, mouse, rat, rabbit, and guinea pig. She found that whereas the larvae developed to maturity in the pig, those fed to the aberrant hosts named did not develop, but encysted in the wall of the alimentary tract. That reencystment of the parasite larvae in aberrant hosts is not a mere zoological curiosity was shown by Cram (*loc. cit.*) who found natural infections of the encysted larvae in the loggerhead shrike, the screech owl, and the red-tailed hawk. In the case of larvae infesting the shrike, Cram demonstrated, by feeding tests, that the worms not only were capable of developing to maturity in the pig when fed to this host, but also were able to reencyst a second time in the wall of the alimentary tract of a wide variety of aberrant hosts including the turkey, chicken, hawk, skunk, opossum, rat, mouse, and blacksnake. Alicata (1931, Jour. Parasitol., 18: 47) also found natural infections of *P. sexalatus* in the brown bat, the worms being encysted in the wall of the alimentary tract, and showed by feeding tests that the larvae in question were able to develop to maturity in the pig.

In spite of the experimental demonstration by Cram that larvae of *P. sexalatus* are able to reencyst in chickens that consume infected beetles, there are, so far as the writers are aware, no published reports of natural infections with this larval nematode in farm-raised fowl. This paper reports the discovery of encysted *P. sexalatus* larvae in farm-raised chickens and briefly summarizes tests bearing on the ability of the larvae to (1) develop in swine, and (2) reencyst or develop in an aberrant mammalian host, namely, the guinea pig.

SUMMARY OF THE INVESTIGATION

During the examination of crops of chickens for *Capillaria*, made by the senior author at the laboratory of the Zoological Division at Chicago, Illinois, third stage larvae of *Physocephalus sexalatus* were found encysted in the walls

of 28 (6.4 per cent) of the 433 crops examined. The numbers of larvae recovered from the individual crops ranged from 1 to 71. Feeding tests with some of these encysted larvae were carried out in Chicago, utilizing 4 guinea pigs, and at Beltsville, Maryland, utilizing 2 9-week old suckling pigs; the latter animals had been raised under conditions known to be effective in preventing extraneous infections with spirurid stomach worms. In tests involving guinea pigs, the encysted larvae in physiologic saline were administered by pipette within a few minutes after removal from the crop wall of the chicken. Encysted larvae selected for swine-feeding tests were placed in physiologic saline and mailed to Beltsville. On receipt at Beltsville, the larvae, which were still encysted, were divided into 4 lots; 2 of the lots were placed in gelatin capsules for feeding, one lot to each pig; one of the remaining lots were fed by pipette to each pig. The guinea pigs were autopsied 25 to 47 days after infection; the entire wall of the digestive tract was examined for encysted larvae by means of press preparations of the tissues, and the contents of the stomach were searched carefully for the worms. The pigs were killed 106 days after infection and the stomachs

TABLE 1.—*Summary of feeding tests with third stage larvae of* Physocephalus sexalatus *recovered from crops of farm-raised chickens*

Animal	Number and source of larvae fed	Days between infection and necropsy[a]	Number and stage of development of *P. sexalatus* recovered
Guinea pig 1*	14, from chicken	44	1 male nearly mature, from stomach
Guinea pig 2**	23, do	45	No worms found
Guinea pig 3***	194, do	47	26 encysted larvae
Guinea pig 4**	11, from guinea pig 3	25	2 do
Pig 1	60, from chicken	106	5 males, 6 females, all mature, 2 immature (sex not determined)
Pig 2	61, do	106	10 males, 7 females, all mature

* Fed larvae twice, the second feeding being 7 days after the first.
** Fed larvae only once.
*** Fed larvae 4 times, over a period of 7 days.
[a] Time intervals refer to time elapsed after last feeding of larvae.

examined for the nematodes. Results of the feeding tests, together with information on the number and source of the larvae fed to each of the test animals, are shown in Table 1.

As can be seen from the table, reencystment of the third stage larvae of *P. sexalatus* in the chicken had not impaired the ability of at least some of the larvae to develop to maturity in the pig. Furthermore, some of the larvae fed to guinea pigs 1, 2, and 3 either became reencysted a second time, or, in the case of guinea pig 1, developed to nearly the mature state. In this connection, it should be pointed out that guinea pig 1 probably had been, for some time, on a deficient diet, which may account for the unusual development of the worm. Presumably the natural immunity of the aberrant host may be responsible for reencystment of the larvae, and it may be possible to break down this immunity through a deficient diet, thereby permitting the worm to develop. In guinea pig 4, which was fed 11 encysted larvae from guinea pig 3, the finding of 2 encysted larvae raises a question as to the number of times the larvae may be able to reencyst in aberrant hosts and still retain their infectivity to swine.

In light of the findings by Cram (*loc. cit.*) that encysted *P. sexalatus* larvae were most numerous in the posterior gut of the shrikes she examined, there may be reason to believe that the actual incidence and intensity of the infections of this larval nematode in farm-raised chickens may be considerably higher than is indicated by our data since, as stated previously, only the crops were examined. Cram suggested that the part played by accidental hosts of these larvae may be looked upon as beneficial from the standpoint of control of this nematode in swine because larvae that become established in such aberrant hosts probably have no opportunity to complete their development in swine. However, as has been pointed out (Spindler, 1942, Yearbook of Agriculture, U. S. D. A., 1276: 752) some of these accidental hosts occasionally may be eaten by pigs at a time when the former harbor the larvae, and the hogs become infected as a result. On the other hand, in view of findings herein reported, it seems reasonable to assume that farm-raised hogs and hogs in garbage-feeding establishments may become infected as a result of the practice of feeding to them the discarded viscera of chickens that are being dressed for human consumption.

Technique for the Beheading and *en face* Examination of Nematodes and Similar Animal Types

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In studies on the taxonomy, anatomy, and morphology of nematodes it has long been known that a close examination of the head and mouth structures as seen from in front, or *en face*, is desirable, and often necessary. The technique for making mounts of such views has been described by workers in the past, but the publications are no longer easily accessible. In 1920 Cobb described a method for obtaining an end view of a nematode, rotifer, or other similar small object (Amer. Micros. Soc. Trans. 39(4): 231–242). A modification of that technique and its value in taxonomic work are discussed by Chitwood and Wehr (1934, Ztschr. Parasitenk. 7(3): 273–335), as applied to the Spiruroidea and other large nematodes generally referred to as vertebrate parasites. To answer the many inquiries received from beginners in the various fields of nematology these techniques are again presented, with certain details and recently developed refinements.

There is every reason to believe that the procedures herein described could be adapted to other zoological or botanical objects. Furthermore, these methods are not limited to the making of *en face* views of heads, but can also be used to advantage e.g. in a study of caudal structure through preparation of ventral, dorsal, or tail-end views, as well as of hand-cut cross sections through any part of the body. With practice, very satisfactory cross sections can be made at great saving of time as compared to the long process of paraffin-embeddings and microtome-sectioning usually employed. The techniques outlined below are those commonly used and recommended by workers in this Division and that of the Zoological Division, U. S. Bureau of Animal Industry.

1. *Fixation:*
   a. *With Flemming (Strong).* (Recommended for very small phytoparasitic, zooparasitic, and free-living nematodes, or those whose cephalic structures are rather indistinctly differentiated. Specimens take a yellow stain.) Pick living nematodes, carefully and without injury, into a drop of water

(physiological salt solution for zooparasites) in a Syracuse watch glass, using a flexible needle. When the required number has been isolated add 1 or 2 drops of the fixative, trying to keep the liquid from spreading. Cover immediately to retain the osmic acid fumes. Let stand, protected from evaporation, from 2 to 18 hours, depending on the depth of color the specimens assume.

b. *With 5% commercial formalin.* (Recommended for medium and large-sized phytoparasitic, zooparasitic, and free-living nematodes. No staining takes place.) Pick living nematodes, carefully and without injury, into a small quantity of the fixative in a Syracuse watch glass, using a flexible needle. No precaution necessary to retain fumes. Keep over night, covered.

c. *With 70% alcohol heated to 80° C.* [Recommended for vertebrate parasites. See A. Looss 1902 and reprint of 1927 (The Sclerostomidae of horses and donkeys in Egypt. Rec. Egypt. Govt. School Med., pp. 25–139)]. Specimens are collected into physiological salt solution where they remain alive and at the same time are cleaned of adhering debris and intestinal contents, for 1 to 3 hours. Fixation then with the heated alcohol will relax and straighten the bodies before killing. In many routine collections it is not possible to heat the alcohol, in which case 70% alcohol at room temperature is used. This fixative also serves as the preservative.

2. *Washing:*

a. *From Flemming (Strong).* Transfer fixed specimens with flexible needle, into an isolated drop of distilled water. Keep tightly covered, for half as long to as long as the fixation time.

b and c. *From formalin and 70% alcohol.* No washing necessary.

3. *Dehydrating:*

a. *From Flemming (Strong).* Make following solution: 1 part pure glycerine, 15 parts distilled water, 4 parts 5% commercial formalin. Pour 15 to 20 cc. of this solution into a standard Syracuse watch glass. Into it transfer the nematode specimens, seeing that they sink to bottom. Place dish away from dust and contamination, where the solution will evaporate gradually in 3 to 5 days. Speed of evaporation to be gauged by worker, depending largely on climatic conditions and room temperature and humidity. Some workers find a calcium chloride or a sulphuric acid evaporation chamber advantageous to facilitate the process. After 3 to 5 days solution should have concentrated to a thin layer of pure glycerine in bottom of watch glass, the specimens lying in it. Test concentration and add additional glycerine.

b. *From formalin.* Same as 3a.

c. *From 70% alcohol.* Make a 5 or 10% solution of pure glycerine in 70% alcohol. Transfer the specimens to a dish containing a quantity of this solution, and place dish away from dust and contamination where the liquid will evaporate gradually in 3 to 7 days. Speed of evaporation to be gauged by worker, depending on permeability of the nematode cuticle, on climatic conditions, and on room temperature and humidity. Some workers find a calcium chloride or a sulphuric acid evaporation chamber advantageous to facilitate the process. Here, penetration and evaporation time may be hastened without danger of tissue shrinkage by cutting these larger specimens some safe distance from the head, such as the anterior portion of the intestine.

After 3 to 7 days solution should have concentrated to a thin layer of pure glycerine in bottom of dish, the specimens lying in it. Test concentration and add additional glycerine.

4. *Beheading:*

Make Kaiser's Glycerin Gelatin to which has been added an extra amount of gelatin. This extra amount will vary according to moisture and temperature conditions of the locality, and will have to be judged by the worker. The consistency of the gelatin mixture, at room temperature, should be "rubbery"; to achieve this stiffness, from ¼ to ½ again as much gelatin must be added to Kaiser's formula.

Heat a small quantity of the glycerine-gelatin in a water bath until it liquefies. Using a clean steel needle, transfer a small drop to the center of a celluloid slide. The drop will stiffen and set immediately. Using a flexible needle, place a glycerine-penetrated specimen in this drop. Re-melt the drop by placing slide on a warming table, remove slide to dissecting microscope, and promptly and gently lower the specimen to the very bottom of the drop. Harden in refrigerator a few minutes.

The beheading is done with a very slender, sharp knife of the type used by surgeons in eye operations. These are manufactured with variously shaped blades; a thin blade 20 to 30 mm. long, especially recommended for the smaller nematodes, causes less distortion of the medium and the operation can be better observed. (Some workers have fashioned such knives from dental instruments ground to the exactly desired shape and fineness, and have found them as satisfactory as the cataract knives.) Under a suitable power of the dissecting microscope, decapitate the nematode squarely, close behind the pharynx or anterior to that point. The point of severance in general can be said to be no farther back than one body-width; for many of the large vertebrate parasites that point is much shallower than a body-width. The cutting is done by a steady pressure rather than a carving stroke, and can be facilitated by steadying the hand against the barrel of the microscope. The need for an exceedingly sharp knife-edge cannot be overemphasized.

Re-melt the drop of glycerine-gelatin on the warming table; transfer the head to another drop of the glycerine-gelatin which has been placed on a clean thin glass slide. Again, lower the head to the bottom of the drop before the glycerine-gelatin stiffens. When working with the smaller nematodes it may be found convenient to place the decapitated body in the same mount with the head, thus keeping the entire specimen together; this can usually be done without interferring with the next step (5).

After some practice it may be possible for the worker to delete the step using a celluloid slide, and to make his cut directly on the glass slide which becomes the final mount. The celluloid slide is used solely for the protection of the delicate knife-edge. Deleting it is especially advantageous with the very small nematodes, where a cut head is easily lost in transfers. The process of beheading may also be somewhat shortened with some of the larger vertebrate parasites of certain shapes, by making the cut in pure glycerine instead of glycerine-gelatin.

5. *Completing mount:*

Select a cover-glass of 0.12 mm. thickness or less, clean it, heat it slightly, and drop it on the center of the medium. Place slide on warming table only long enough to melt the gelatin out to the edge of the cover-glass. The quantity of glycerine-gelatin per size of cover-glass is important, and can be

judged through practice. The mount should be slightly thicker than an ordinary mount, to encompass the head turned up on end without flattening it. Immediately transfer the slide to the compound microscope, locate the cut head, and with a stiff needle gently shove the edge of the cover-glass slightly back and forth until the head is seen to be on end. Shifting to a 4 mm. objective may be necessary to make a "trued" or symmetrical view of the mouth opening and cephalic structures. If the glycerine-gelatin hardens before such a trued view can be effected, return the slide to the warming table, barely re-melt the gelatin, and try again.

If the head fails to turn *en face*, the cause may be attributed to two main faults: a) Not enough glycerine-gelatin was used per size of cover-glass, b) Cut was too deep to bring the head up to desired position.

Allow the slide to remain on the microscope stage until the gelatin has set, watching it from time to time to see that the position of the object has not changed. It may be further chilled by placing in refrigerator before examining under the oil immersion objective.

Ring Nematodes (Criconematinae). A Possible Factor in Decline and Replanting Problems of Peach Orchards

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While making a survey of root-knot nematodes in peach orchards, Dr. C. N. Clayton of the North Carolina State College of Agriculture and Engineering called our attention to certain small lesions on the roots of peach trees. Examination of such roots from several orchards in the vicinity of Candor, N. C., disclosed large numbers of a species of ring nematode, *Criconemoides simile* (Cobb 1918) n. comb. Further sample materials disclosed some 10,000 specimens from only 200 grams of root.

Various old peach orchards examined in North Carolina all contained one or two types of root-knot nematode. Many of these orchards had shown declines in growth and yield and what is called "winter injury," neither of which is entirely attributable to senescence. When such orchards are replanted the young plants commonly grow very poorly. Such case histories are generally attributed to long term build-up of the root-knot population, but we doubt that this is the complete answer.

Recently Dr. Leon Havis of the Division of Fruit and Vegetable Crops and Diseases brought to our attention some samples of peach roots from an old orchard near Salisbury, Maryland. These root samples contained *Criconemoides simile* in large numbers but no root-knot nematodes. The latter have not been observed as a problem in peach orchards anywhere in Maryland, yet growers have found that when old orchards are replanted the young plants show poor growth. "Winter injury" is encountered in Maryland as well as North Carolina. Similar symptomatology in the absence of root-knot has caused some investigators to feel the problem may be one of minor elements or fertilizer exhaustion but no conclusive experimental work has been done on this theory. Soil problems are seldom simple, and many organisms and environmental conditions may cause similar disease syndromes. Ordinarily, nematodes of the *Criconemoides* group live in sandy soils as parasites of woody plants, but their pathological importance has not been properly investigated. However, it is worth noting that a similar nematode,

Cacopaurus pestis, was found by Thorne (1943, Proc. Helm. Soc. Wash. v. 10(2): 78–83) to be involved in slow decline of walnut groves. In the present instance, abundance of these organisms causes one to suspect that they may indeed play a part in peach orchard decline.

The nematode associated with this problem may be defined as follows:

Criconemoides simile (Cobb 1918) n. comb.

Synonym: Iota simile Cobb 1918 (Contrib. Sc. Nemat. No. 7: 204, Waverly Press, Baltimore).

Diagnosis.—Small nematodes, adult females 550–650 μ long, 40 to 50 μ wide, body blunt at both ends, very heavily annulated, margins of body crenate, annules 90 to 108 in number, posterior edge of annules finely marked longitudinally in larvae, smooth in adult, 1 or 2 annular anastomoses. Stylet 70 to 75 μ long, hilt and knobs 17 μ long, knobs of stylet 3.5 to 4.5 μ long; width across knobs 11 to 12 μ, knobs very slightly reflected anteriad. Corpus of esophagus elongate, approximately 35 × 21 μ; isthmus and bulbar region 30 μ long with a maximum diameter of 14 μ. Excretory pore at 31st to 33rd annule from head. Vulva on seventh annule from tail observed in ventral view; vulva complicated with two ventrolateral rounded posteriorly projecting lips. Anus round, minute, on posterior side of fifth or sixth annule from tail; tail a rounded button.

The Occurrence of *Platynosomum illiciens* (Braun, 1901) in a North American Hawk.

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A series of 160 hawks of 12 species from ten states and the Province of Manitoba has been examined for dicrocoeliid trematodes. Only one species of dicrocoeliids was found in them indicating the infrequency with which North American hawks are infected with trematodes of this group.

Of the 160 hawks examined 4 were broad-winged hawks. *Buteo platypterus,* 2 of which, collected in Ohio and Wisconsin, harbored in their bile ducts 3 and 8 specimens respectively of a trematode of the genus *Platynosomum.* These specimens, after comparison with both the original and the recently expanded description by Travassos (1944, Monogr. Inst. Oswaldo Cruz, No. 2, 357 pp.) of *P. illiciens* (Braun, 1901), have been assigned to that species. Since this is the first time this trematode has been reported from a host outside of Brazil, and in order to help delimit the variation within the species, a description of this material is presented.

The 8 specimens from one hawk, though sexually mature, had not attained their maximum growth. After fixation they measure only 2.69–2.87 mm. long by 0.80–0.93 mm. wide, about half the size of the fully mature specimens from the second hawk. However, there is no question that they belong to the same species as the larger worms since both agree in shape of body, ratio of sucker sizes and position of acetabulum, length of ceca, shape and relative positions of gonads, position of genital pore and extent of vitellaria, characters which are more significant in identifying dicrocoeliids than simple measurements. The measurements of two of the larger worms are given in the description that follows.

Body (Fig. 1) fairly thick and muscular, slightly contracted, 5.10–5.38 mm. long by 1.95–1.96 mm. wide; widest near anterior end of vitellaria. Cuticle thick, without spines. Oral sucker subterminal, 0.420 mm. long by 0.395–0.434 mm. wide. Acetabulum large, muscular, with deep cup-shaped lumen, 0.495–0.546 mm. long by 0.580–0.585 mm. wide, situated in anterior third of body. Ratio of width of oral sucker to acetabulum 1: 1.33–1.48. Prepharynx absent. Pharynx

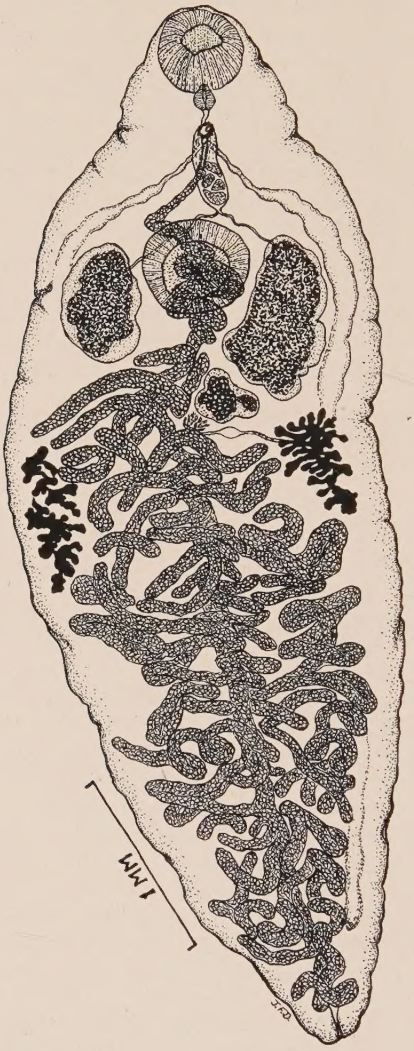

FIG. 1. *Platynosomum illiciens* (Braun, 1901), ventral view.

globular, muscular, 0.120–0.127 mm. long by 0.107–0.111 mm. wide. Esophagus thin-walled, fairly wide, approximately 0.150–0.160 mm. long, bifurcating about midway between suckers. Ceca thin-walled, narrow and slightly wavy in young specimens, wider and more voluminous in older ones, passing lateral to testes, dorsal to inner margins of vitellaria to terminate just a short distance from posterior end of body. Excretory pore terminal; remainder of system not observed.

Genital pore median, located ventral to intestinal bifurcation in relaxed specimens, about midway esophagus in specimens with contracted anterior end. Testes elongated oval in shape with smooth to slightly irregular margins in young specimens, with more irregular to lobed margins in old specimens, 0.602–0.868 mm. long by 0.462–0.520 mm. wide, situated directly opposite so that from one-third to one-half of their anterior extremity lies within acetabular zone. Vasa efferentia arising from dorsomedial surfaces of testes and passing antero-medially to unite as they enter cirrus sac. Cirrus sac elongated oval, 0.266–0.448 mm. long by 0.137–0.154 mm. wide, containing a much convoluted seminal vesicle, ejaculatory duct, a few prostatic gland cells and eversible cirrus. Cirrus sac with posterior end touching acetabulum in young specimens or situated slightly in front of it in old specimens. Ovary distinctly lobed, equal in size to testes in young specimens, from one-half to one-third size of testes in old specimens, 0.266–0.350 mm. long by 0.336–0.350 mm. wide, situated immediately postero-mesially to either left or right testis; behind left in 4 specimens, behind right in 5. Seminal receptacle relatively small, approximately 0.072 mm. in diameter, lying dorsal to mesial half of ovary. Mehlis' gland small and diffuse, situated in midline at posterior level of ovary. Laurer's canal not observed. Vitellaria composed of numerous irregular follicles joined together in dendritic masses, 0.60–1.02 mm. long, occupying zone immediately posterior to ovary. Vitelline ducts arising about one-third distance from anterior end of vitellaria. Uterus much convoluted, filling most of body posterior to gonads, then passing between testes and dorsal to acetabulum where it forms 2–3 lateral loops before ascending to genital pore by undulating course. Mature ova numerous, dark brown, 41–47 μ long by 26–31 μ wide.

A specimen, No. 46378, has been placed in the Helminthological Collection of the U. S. National Museum.

A Note on *Arthrocephalus lotoris* (Schwartz, 1925) Chandler, 1942 and other Roundworm Parasites of the Skunk, *Mephitis nigra*.

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During the winter of 1947–1948, the authors had the opportunity to examine postmortem 5 skunks which, during their nocturnal maraudings, had met with accidents leading to their deaths. Each of these animals harbored some hookworms in the small intestine. Erickson (1946, Am. Midland Naturalist, 36: 494–509) apparently found no hookworms in the skunks examined by him and cites only one previous report of the occurrence of such nematodes in this host, namely, that of Dikmans (1931, Journal of Parasitology, 18: 56) who reported finding *Uncinaria* sp. in the small intestine of the skunk, *Mephitis mesomelas mesomelas*, at Jeanerette, Louisiana. An examination of the host-parasite records in the Zoological Division, Bureau of Animal Industry, has failed to disclose any other reports of the occurrence of hookworms in these animals.

On examination the above mentioned hookworms were found to be identical with *Arthrocephalus lotoris* (Schwartz, 1925) Chandler, 1942. This worm was originally described by Schwartz (1925, Proceedings U. S. National Museum, v. 67, art. 26, pp. 1–4) as *Uncinaria lotoris* n. sp. from the raccoon, *Procyon lotor*, in Maryland. It has since been reported from this host in Michigan, Illinois and Texas and was recently recovered from it at Tifton, Georgia. Chandler (1942, Journal of Parasitology, 28: 255–268) transferred this nematode to the genus *Arthrocephalus* Ortlepp, 1925, because it resembled the other members of

this genus in having the buccal capsule divided by sutures into a number of cuticular plates. He reported, however, that *A. lotoris* differed from *A. gambiensis* Ortlepp, 1925 (Journal of Helminthology, 3: 151–156) in possessing a pair of obtuse ventral lancets and in the absence of a dorsal cone.

Examination of some specimens of *A. gambiensis* collected from a mongoose in Africa and presently deposited in the U. S. National Museum Helminthological Collection, shows that these nematodes possess a dorsal cone as described and figured by Ortlepp and that there are no teeth or lancets in the buccal capsule. However, examination of specimens of *A. lotoris* from raccoons in Maryland and Georgia, including the paratypes, and from skunks in Maryland, shows that these nematodes do possess a distinct dorsal cone or gutter, as mentioned by Schwartz in his original description of the species. An additional interesting feature is that in the specimens from both raccoons, again including the paratypes, and skunks, both the base and the shaft of the dorsal cone bear

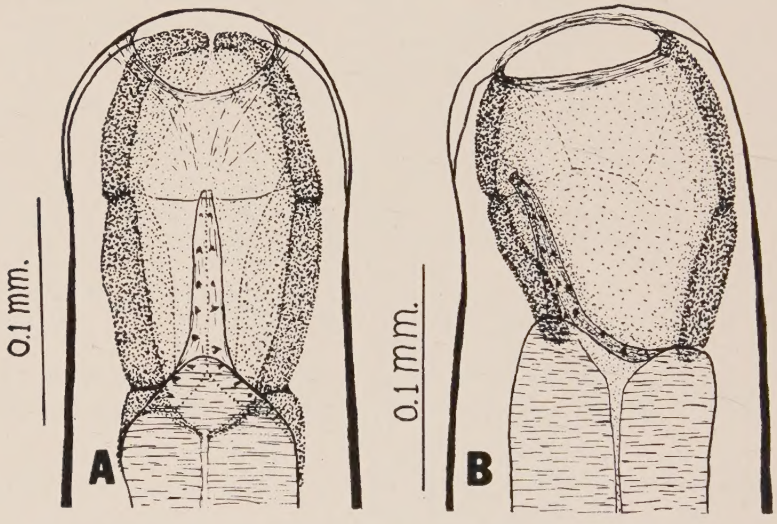

FIG. 1. Head end of *Arthrocephalus lotoris* showing dorsal cone. A—Dorsal view. B—Dorsal oblique view.

a number of well marked small spines. Apparently Vaz [1935, Redescrição de *Arthrocephalus maxillaris* (Molin, 1860) necatorineo parasita de *Procyon cancrivorus*, Rev. de Biol. e Hygiene, 6: 9–12] observed similar structures on what he terms the "glandular duct"[1] leading from the esophagus into the buccal capsule of *A. maxillaris*. While this author does not specifically mention such spines, it may reasonably be inferred from an examination of Fig. 1 of his paper that he noted some structures in those locations.

Arthrocephalus lotoris (Schwartz, 1925) Chandler, 1942, therefore, differs from *A. gambiensis* Ortlepp, 1925, principally in two features, namely, the presence of a pair of obtuse ventral lancets in the buccal cavity and the greater length of the spicules, those of *A. gambiensis* being 0.360 mm. long and those of *A. lotoris* measuring from 0.700–0.800 mm. *A lotoris* differs from *A. maxillaris* only in the presence of ventral lancets in the buccal cavity, *A. maxillaris* showing, according to Vaz, neither lancets nor teeth in the buccal capsule.

[1] For the purpose of the present paper the terms dorsal cone (Ortlepp), dorsal gutter (Schwartz) and glandular duct (Vaz) are considered to be synonymous.

Key to species of Arthrocephalus

1.	Ventral lancets present in buccal capsule	*A. lotoris*
	Ventral lancets absent	2
2.	Spicules 0.0360 mm. long	*A. gambiensis*
	Spicules 0.850 mm. long	*A. maxillaris*

The hookworm collected from the skunk at Jeanerette, Louisiana, previously identified as *Uncinaria* sp., has been re-examined and re-determined as *Arthrocephalus* sp. because the buccal capsule is divided into cuticular plates. There is in the U. S. National Museum Helminthological Collection another female specimen of hookworm collected from a skunk in the Pisgah National Forest. It was identified as *Uncinaria lotoris?* Examination shows it to be a specimen of *Arthrocephalus.*

Other nematodes found in these skunks were *Skrjabingylus chitwoodorum, Physaloptera maxillaris, Ascaris columnaris, Molineus patens* and *Capillaria* sp. Some of the specimens of *Skrjabingylus chitwoodorum* are larger than any previously reported. Hill (1939, Journal of Parasitology, 25: 475–478) who originally described this species, reported the average length of female specimens to be about 26 mm. and that of the males 15 mm. The average lengths of the female and male cotypes, deposited in the U. S. National Museum Helminthological Collection, are 32 mm. and 16 mm. respectively. Goble (1942, Journal of Mammalogy, 23: 96–97) reported the average length of the female and male specimens of this nematode collected by him from *Mephitis nigra* in New York to be 35 mm. and 20 mm. respectively. Some female specimens collected from one of the skunks necropsied at the Agricultural Research Center, measure 56.5 mm. and some male specimens 29 mm. in length. The average length of the spicules of 5 specimens of Hill's cotypes is 0.680 mm., range 0.647 to 0.724 mm., that of Goble's specimens (loc. cit.) 0.780 mm., range 0.715–0.830 mm., that of two specimens collected at A.R.C. 0.820 mm., one measuring about 0.840 mm. and the other 0.800 mm.

The Ascaricidal Action of Reduced Dosages of Sodium Fluoride in Swine and the Influence of Various Concentrations of the Chemical on Feed Consumption

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Since the report by Habermann, Enzie, and Foster (Amer. Jour. Vet. Res. 6: 131–144) in 1945 on the anthelmintic action of sodium fluoride in swine, the chemical has been extensively used for the removal of large roundworms from these animals. Essentially, the treatment consists in feeding the chemical for 1 day in dry, ground feed at a concentration of 1 per cent by weight. This regimen has proved to be very successful, yet, as emphasized by us in a recent review (Foster, Enzie, Habermann, and Allen, 1948, Amer. Jour. Vet. Res. 9: 379–385), there was need for further study to determine the optimum rate of administration. The literature on the treatment and a consideration of the problem of determining the best dosage were fully covered in the aforementioned review. Data are presented herein on the efficacy of feed mixtures containing less than 1 per cent sodium fluoride and on the influence of various concentrations of the chemical on feed consumption.

PROCEDURE

In most instances, the methods employed were the same as those described in previous articles (q.v.). There was a slight departure from the usual technic, however, in trials on the influence of various concentrations of the chemical on feed consumption. Pigs of different weights were used with each concentration of sodium fluoride tested; but for evident reasons, pen mates were carefully selected in order to obtain groups of pigs of uniform size, weight, and vigor. The animals were confined in concrete-floored pens for 3 days and given regular feed *ad libitum* in a self-feeder. The average daily feed consumption per pig was determined over a period of 2 days, and on the third day the average consumption per pig of medicated feed mixtures was determined. The pigs were returned to regular feed on the fourth day. The feces were collected daily throughout the test period and examined for parasites. On the eighth or ninth day after treatment, the animals were slaughtered and examined for parasites and lesions.

RESULTS

A total of 61 pigs, treated individually or in groups of 2 to 30 animals, were given 0.75 per cent sodium fluoride in dry, ground feed for 1 day (Table 1). In the aggregate, the treatment removed 97 per cent (187) of 192 ascarids, 76 per cent (127) of 166 stomach worms (*Ascarops strongylina*), 20 per cent (260) of 1249 nodular worms, and 3 per cent (9) of 283 whipworms. Vomitus was noted in 1 pen of 2 pigs. In another group, 1 runt died on the second day after treatment; at necropsy, the entire gastrointestinal tract was empty, but no significant lesions were observed. It was doubtful that the pig consumed any of the medicated feed, and death was attributed to general debilitation. Although some of the pigs were not infested with large roundworms, the tests with these animals are of value in that they add to the available information on the tolerance of swine for sodium fluoride.

In 2 groups of pigs, containing 14 and 20 animals, respectively, the dosage was reduced to 0.5 per cent of the feed for 1 day. The treatment removed, in the aggregate, only 54 per cent (86) of 158 ascarids from the test animals. It is noteworthy, however, that 58 of the 72 ascarids recovered at necropsy were found in 1 pig. One animal vomited but the others were unaffected by the treatment.

It should be noted that the efficacies obtained against ascarids in the several tests are probably conservative because there was evidence that undetermined numbers of these worms were pilfered by rats. The data are admittedly limited, but they indicate that the 0.75 per cent concentration of the drug is superior to the 0.5 per cent mixture.

Rather marked reductions in feed consumption were noted when various concentrations of sodium fluoride were given in the feed (Table 2). Sixteen pigs, averaging 42.4 pounds each, were used in trials with a 1 per cent mixture of the chemical. When the pigs were given the medicated mixture, feed consumption was reduced to approximately 43.4 per cent of the norm. No untoward reactions were noted during the test period, and no significant lesions were found at necropsy.

A mixture containing 0.75 per cent by weight of sodium fluoride was given to 20 pigs averaging 35.6 pounds body weight. With this concentration, the pigs consumed 49.1 per cent of the amount of feed normally consumed in one day. Vomitus was noted in 1 pen of 6 pigs after treatment. Nephritis, enteritis, and/or slight to moderate gastritis were noted in some of the pigs at autopsy.

Sixteen pigs, averaging 51.4 pounds each, were used in trials with a mixture containing 0.5 per cent by weight of sodium fluoride. At this concentration feed

TABLE 1.—*Data on anthelmintic action of sodium fluoride administered to swine for 1 day as 0.5, 0.75, and 1.0 per cent mixtures of the feed.*

Percentage of Sodium Fluoride	No. of Pigs	Parasites	Removed	Left	Efficacy (per cent)	Remarks
1.0	182	Ascarids	926	68	93	Summary published data (See Foster, et al. 1948)
	1	None	.....	.....	.....	
	2	Ascarids	38	0	100	
		Nodular worms	0	158	0	
	2	Ascarids	7	1	87	
		Nodular worms	0	57	0	
		Whipworms	0	9	0	
	2	Ascarids	?	0	?	Ascarid eggs on fecal exam. before treatment, vomitus in pen after treatment.
		Nodular worms	0	5	0	
		Whipworms	0	16	0	
	2	Stomach worms	19	5	79	
		Nodular worms	93	14	86	
	3	None	.....	.....	.....	
0.75	7	Whipworms	0	7	0	1 runt died on 2nd day after treatment, see text.
	2	Ascarids	15	0	100	
		Stomach worms	86	28	75	
		Nodular worms	50	63	44	
	2	Ascarids	25	0	100	
		Stomach worms	22	6	78	
		Nodular worms	48	133	26	
	8	Ascarids	4	0	100	
		Nodular worms	51	317	13	
		Whipworms	3	61	4	
	30	Ascarids	98	4	96	
		Nodular worms	18	242	6	
		Whipworms	6	181	3	
	61	Ascarids	187	5	97	
		Stomach worms	127	39	76	
		Nodular worms	260	989	20	
		Whipworms	9	274	3	
0.5	14	Ascarids	23	60	27	1 pig vomited; 58 ascarids in 1 pig at autopsy.
	20	Ascarids	63	12	84	
	34	Ascarids	86	72	54	

consumption was reduced to 59.2 per cent of the norm. There were no untoward reactions to the treatment, and no significant lesions were found at autopsy.

In these trials, the animals were maintained on full feed until treated, and the amount of medicated feed consumed was probably diminished accordingly.

TABLE 2.—*Data on the influence of various concentrations of sodium fluoride on feed consumption.*

Percentage of sodium fluoride	No. of pigs	Average weight (pounds)	Feed consumed daily before treatment (Gm./pig)	Medicated feed consumed		Sodium fluoride consumed		Ascaricidal efficacy (percent)	Remarks
				Gm./pig	Percent norm.	Gm./pig	Gm./lb.		
	6	20.25	455.4	170.3	37.3	1.703	0.085	94(34/36)	
	2	99.5	2476.0	940.0	37.9	9.400	0.094	91(118/129)	
1.0	4	59.5	1921.8	1067.5	55.5	10.675	0.179	98(53/54)	
	4	30.0	585.0	133.2	22.7	1.332	0.044	.....	
	16	42.4	1106.9	481.5	43.4	4.815	0.113	93(205/219)	
	6	26.9	891.6	279.1	31.3	2.093	0.077	88(93/105)	Vomitus in pen
	2	94.0	3502.0	1513.0	43.2	11.347	0.120	91(44/48)	Kidneys hemorrhagic; marked congestion of small intestine; petechial hemorrhages in fundus of stomach.
0.75	8	21.5	355.0	266.1	74.9	1.995	0.092	54(58/106)	48 ascarids at autopsy were in 1 pig.
	4	47.75	1371.0	832.5	60.7	6.244	0.130	100(9/9)	Kidneys hemorrhagic in 3 pigs; fundus of stomach hemorrhagic in 1 pig.
	20	35.6	1033.8	508.0	49.1	3.81	0.106	76(204/268)	
	6	42.5	1575.0	835.0	53.0	4.175	0.098	94(16/17)	
	1	110.0	4669.0	2481.0	53.1	12.405	0.112	94(54/57)	
0.5	4	41.4	1208.3	885.0	73.2	4.425	0.106	87(48/55)	
	3	88.0	2266.0	1462.0	64.5	7.310	0.083	90(20/22)	
	2	14.0	473.0	197.0	41.6	0.985	0.070	100(5/5)	
	16	51.4	1668.5	988.1	59.2	4.940	0.096	91(143/156)	

Note: There was no underfeeding before treatment.

On this account, the data are probably not fully representative of the ascaricidal action that would have been obtained if the usual practice of slightly underfeeding the day before treatment had been followed. Moreover, as noted previously, undetermined numbers of worms were removed from the pens by rats. For these reasons, the data do not permit significant comparisons of ascaricidal action among the groups and probably should be disregarded in calculating the aggregate efficacies of the various mixtures.

DISCUSSION

In experimental and field trials, sodium fluoride has proved to be a safe and effective treatment for the removal of large roundworms from swine when given as a 1 per cent mixture of the feed for 1 day. The usefulness of lower concentrations, however, has not been adequately explored. Consequently, in trials reported herein the chemical was given to swine in dosages of 0.75 and 0.5 per cent of the feed for 1 day. The lower dosage appeared to be definitely inferior to the 1 per cent regime although the poor aggregate efficacy was attributable largely to the results on a single animal. This attests, nevertheless, the variable efficacy of the chemical at this concentration. At the 0.75 per cent level, on the other hand, the aggregate efficacy compared very favorably with that reported for the 1 per cent dosage. This would indicate that the minimum effective dosage is approximately 0.75 per cent.

In establishing the optimum dosage, however, coordinate consideration must be given to the safety factor, and, paradoxically, a reduction in the concentration of sodium fluoride might diminish the safety of the treatment beyond the economy which would accrue from the use of smaller amounts of the chemical. It has been noted previously, for example, that at the 1 per cent concentration, the pigs did not eat sufficient amounts of the mixture to incite toxic reactions other than occasional vomiting and soft feces. In most instances, however, the animals consumed and retained enough of the chemical to produce highly satisfactory ascaricidal action. With this regime, therefore, there was a self-limited consumption which seemed to afford some measure of protection against serious intoxication. This self-limited consumption was associated with the unpalatability of the mixture since non-medicated feed was usually eaten readily.

It is conceivable, moreover, that with smaller concentrations of the chemical in the feed, larger absolute dosages of sodium fluoride might be obtained before the unpalatability factor became operative. In feeding trials with concentrations of 1.0, 0.75, and 0.5 per cent sodium fluoride, it was found that although feed consumption increased when the concentration of the chemical was reduced, the amount of sodium fluoride ingested per pound of body weight diminished. It was noted, however, that marked variations occurred at all dosage levels, and as a consequence, correlations between the concentration of sodium fluoride in the feed and the amount of the chemical ingested per pound of body weight were not evident. It appears, then, that the palatability of the different mixtures is subject to individual variation.

In all, 1 death occurred among the 147 pigs employed in the tests herein described, but as noted elsewhere (*vide supra*), the loss was attributed to factors other than intoxication from the drug. Five of the remaining 146 animals (less than 4 per cent) showed lesions ascribable to fluorine intoxication; but for the most part, the unpalatability of the various mixtures appeared to protect the animals against serious intoxication from overindulgence. The lesions attributable to fluorine intoxication were found only in animals on the 0.75 per cent regime, but the information is too limited to assay the relative toxicities of the different mixtures.

The data on the efficacy of mixtures containing 0.75 per cent sodium fluoride show sufficient promise to warrant additional trials with this dosage. The findings are, moreover, of some practical significance. They show that a dosage of 0.75 per cent is essentially neither better nor worse than that of 1.0 per cent; that either dose rate provides a maximum of safety and efficiency, and that there is, therefore, the practical advantage of some latitude in dosage. There is, however, little, if any, reason to warrant recommending a change in the currently employed dosage of 1.0 per cent, although this concentration need not, and consequently should not, be exceeded.

SUMMARY

In trials involving 61 pigs, feed mixtures containing 0.75 per cent sodium fluoride removed 97 per cent (187) of 192 ascarids, but only relatively insignificant numbers of other gastrointestinal parasites. This efficacy against large roundworms compares very favorably with the published record for the currently recommended dosage of 1.0 per cent. One pig died during the course of the investigations, but the animal was a poor risk for medication and death was attributed to general debilitation. With the exception of vomition in one instance, the rest of the animals were unaffected by the treatment. Concentrations of 0.5 per cent of the chemical were comparatively ineffective in trials with 34 animals.

Investigations on the influence of various concentrations of sodium fluoride on feed consumption showed that mixtures containing 0.75 per cent of the chemical were not significantly more palatable than those containing 1 per cent of the drug. This indicated that the natural protection against intoxication afforded by the self-limited consumption of 1 per cent mixtures would probably prevail with the lower concentrations as well. The findings did not warrant a change in the currently recommended dosage of 1 per cent, but they showed that some latitude in dosage was possible without affecting significantly the safety or efficacy of the treatment.

Some Parasites of the Wild Turkey (*Meleagridis gallopavo silvestris*) in Maryland

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At the request of Mr. Henry P. Bridges, Secretary-Treasurer of the Woodmont Rod and Gun Club, Hancock, Maryland, the writers visited, in July, 1948, the wild turkey flock maintained by that Club. They had been informed earlier that a number of the birds were sneezing and coughing and that gapeworms (*Syngamus trachea*) were suspected as being the cause of the trouble.

On our arrival at the Club, none of the birds were found to be showing symptoms typical of gapeworm infestation. However, several of them were suffering from sinusitis, some rather severely. A small sample of fecal material was brought back to the laboratory at Beltsville, Maryland, and examined for parasite eggs and larvae. Only a few capillarid eggs were found.

Early in August, 1948, Mr. Bridges kindly consented to ship to the writers for post-mortem examination two of his most severely affected birds. These birds were in poor condition and were suffering severely from sinusitis when they arrived. They were kept under observation for several days, during which time daily examinations were made of their fecal discharges; no gapeworm eggs were recovered. However, considerable numbers of capillarid and trichostrongyle eggs were found, the latter mostly in the 32- and 64-cell stages of development.

On August 23 the two birds were necropsied and were found to be infested with tapeworms of three different species, trichostrongyles, ascarids, and capillarids. There were no gapeworms in either bird. As all the parasites recorded were present in large numbers, it is probable that their presence contributed to reducing the natural resistance of the birds to the sinus condition that was evidently the immediate cause of their unthriftiness.

Since little information is available in print regarding the internal parasites infesting the wild turkey, and since four of the parasites found in the two birds examined post-mortem had not been previously reported from wild turkey, so far as the writers could ascertain, this article is intended to acquaint interested persons with some of the species that, on occasion, occur in the wild turkey of the eastern part of the United States.

TAPEWORMS

The three species of tapeworms found, all occurring in the intestine in considerable numbers, were identified as *Raillietina williamsi* Fuhrmann, 1932, *Raillietina cesticillus* (Molin, 1858), and *Davainea meleagridis* Jones, 1936.

Raillietina cesticillus is one of the commonest species of tapeworms occurring in poultry, including the chicken, domestic turkey, guinea fowl, and bobwhite quail. Its presence in the wild turkey, though previously unreported, is therefore not surprising. It is a fairly large parasite, attaining a length of as much as 12 centimeters, and occurs in the duodenal and jejunal regions of the digestive tract. House-flies and various species of ground and dung beetles serve as intermediate hosts, the birds acquiring the parasite through swallowing the infested insects.

Raillietina williamsi Fuhrmann, 1932 (syn.—*Davainea fuhrmanni* Williams, 1931) has previously been found in wild turkeys in Pennsylvania on two occasions (Williams, 1931, and Wehr and Coburn, 1943). This species has not, apparently, been reported from any other host. *R. williamsi* is a relatively large parasite, attaining a length of 36.7 centimeters, and occurs in the small intestine of its host. *Raillietina silvestris*, a new name proposed by Jones (1933) for this species, is a synonym of *R. williamsi* Fuhrmann, 1932.

The discovery of *Davainea meleagridis* in the two wild turkeys was of considerable interest for the reason that this species, so far as could be ascertained, has been reported previously only once, by Jones (1936), who named and described it. Jones found it in a domestic turkey purchased on the Washington, D. C., market, the bird, presumably, having been raised in near-by Maryland or Virginia. *D. meleagridis* is a very small parasite, reaching a length of only about five millimeters.

Nothing is known of the life histories of the last-named two species, but it is safe to asume that both have intermediate hosts among small invertebrates.

NEMATODES

Numerous trichostrongyles, *Trichostrongylus tenuis* (Mehlis, 1946), were found in the ceca. These are small, slender roundworms which may effect the bird host rather seriously when present in large numbers. The ceca of both turkeys were greatly thickened, highly inflamed, and reddened. Extensive destruction of the mucosa was present, due to the burrowing and blood-sucking habits of these parasites.

Trichostrongylus tenuis has a direct life-history. The eggs are passed in the droppings of the host, and hatch within 36 to 48 hours after being passed. Within approximately two weeks the larvae reach the infective stage, having

molted twice within this period. When eaten by a susceptible bird host, they complete their life history by molting twice more within the ceca, after which they are mature and ready to renew the developmental cycle by producing eggs. Since it was determined by Cram and Wehr (1934) that *T. tenuis* and *T. pergracilis* (Cobbold, 1873) are synonymous, the list of hosts of this roundworm has been significantly augmented. Among the many birds parasitized by this species in America and Europe are the chicken, turkey, guinea fowl, duck, goose, Canada goose, bobwhite quail, pheasant, red grouse, European partridge, and great bustard. The present report appears to be the first listing of *T. tenuis* from the wild turkey.

Great numbers of small, unidentifiable, immature ascarids were found in the duodenum. These were presumed to be larval forms of *Ascaridia dissimilis*, a species reported from wild turkeys in Georgia and Pennsylvania. This species has a direct life-history which is very similar to that of *Trichostrongylus tenuis*, the eggs being passed in the droppings and becoming infective within two to three weeks. When swallowed by a susceptible host they hatch, and the worms reach maturity in about two months.

Large numbers of capillarids, *Capillaria longicollis* Rudolphi, 1819 (syn.—*Capillaria caudinflata* (Molin, 1858)), were present in the small intestine. Previously these worms have been found occasionally in chickens, domestic turkeys, and pheasants, but apparently not in the wild turkey. Baker (1930) noted that these parasites were associated with ulcerous patches varying in size from pinpoint areas to greatly extended and hardened areas. An infested chicken observed by Graham, Thorp, and Hectorne (1929) in Illinois showed weakness, anemia, and emaciation before death. At necropsy, the intestine just anterior to the ceca was markedly dilated, with a follicular diphtheritic enteritis present. Owing to the presence of other species of parasites in the intestines of the two wild turkeys examined by the writers, it was not possible to determine the nature and extent of the pathology ascribable to this species. *Capillaria longicollis* requires an earthworm intermediate host for its complete development.

MITES

In addition to the internal parasites discussed above, the two wild turkeys were found to be harboring a feather mite, *Megninia cubitalis* Megnin (identified by E. W. Baker, Bur. Ent. and Plant Quarantine). The mite, however, was not present in sufficient numbers to cause any noticeable injury to the plumage of the host.

CONTROL

The most practical control measures against tapeworms and most of the nematodes affecting poultry and game birds are sanitary measures. The selection of well-drained soil, of a gravelly or sandy nature, on which to locate the runs for the breeding stock, and the rotation of the young stock, if possible, between two or more runs, changing the birds from one run to another every two or three months, should be of considerable value in keeping down infestations with these parasites. The runs that are not in use may be prepared and planted to some green crop, left lie fallow, or simply left undisturbed. When the runs are thus vacated by birds for a period, many of the immature stages of the parasites in the soil will die. The house and near-by grounds should be cleaned as often as possible, and the droppings and litter disposed of in such a way that the birds cannot gain access to them. In parasites such as tapeworms and *Capillaria longicollis*, which require intermediate hosts, complete control is greatly complicated if not rendered impossible. However, since most of these parasites are

comparatively harmless unless present in large numbers, any measures that tend to reduce their numbers to any marked extent can be considered reasonably effective.

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Studies on Bovine Gastro-intestinal Parasites. XIV. *Haemonchus contortus* Immunity Experiments with Injections of Dried Worm Powder and Immune Blood Serum

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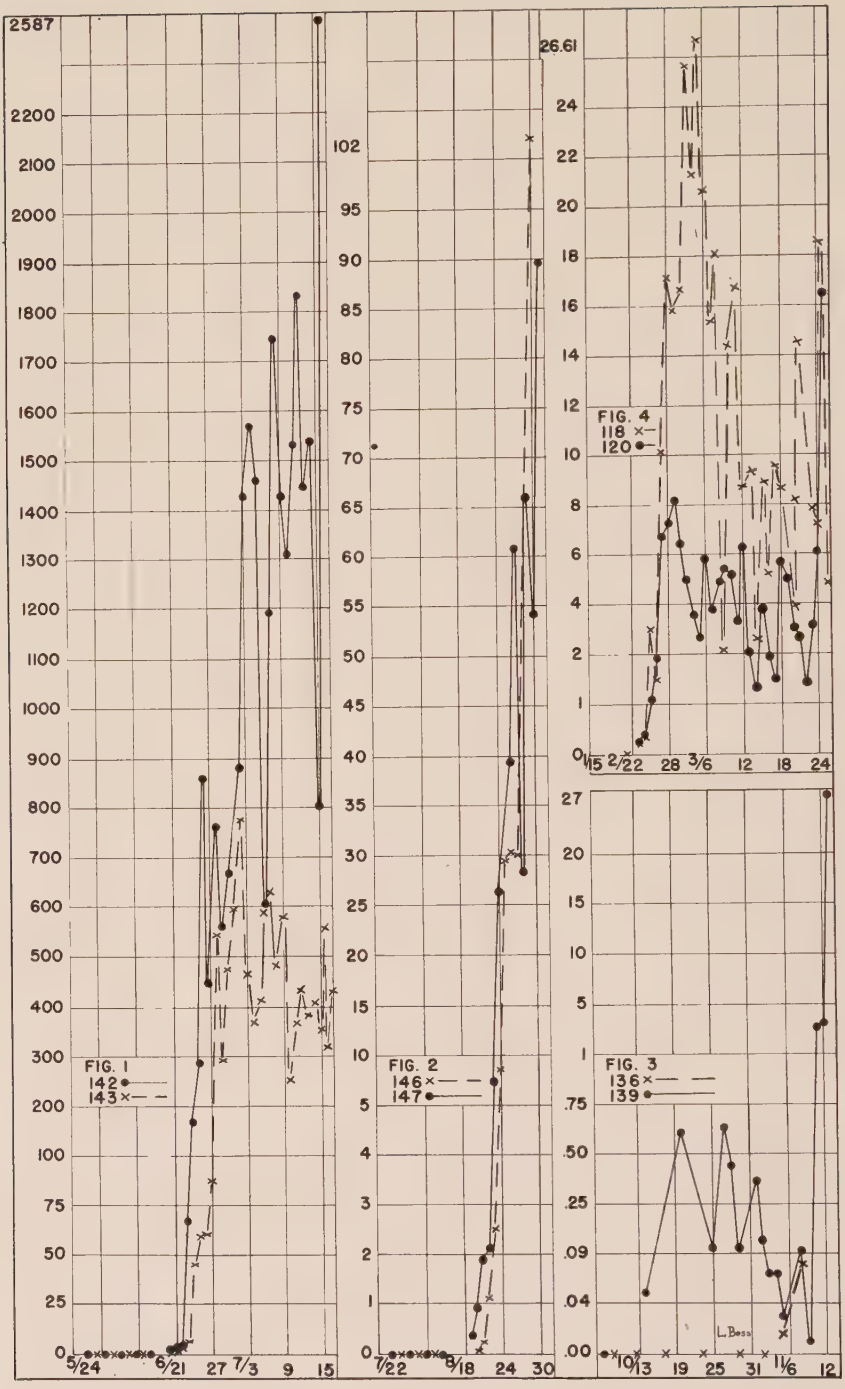
Some reports of experiments designed to develop an active immunity to parasites and to transfer an existing immunity passively are to be found in the literature. Miller (1931, 1932) demonstrated that a very definite active immunity to *Cysticercus fasciolaris* was developed by periodic intraperitoneal injections of fresh or powdered worm material of the adult form of this tapeworm, *Taenia taeniaformis*. Miller (1932, 1934) also demonstrated the passive transfer of immunity against *Cysticercus fasciolaris* by intraperitoneal injections of serum from immune and from infected rats. Kerr (1938) failed to induce an artificial immunity against the dog hookworm and pig ascaris. Kerr and Petkovich (1935) secured a much lower infection of *Fasciola hepatica* following injections of dried powdered fluke material in rabbits. The writer (Mayhew, 1944) failed to develop any protection in calves against the large stomach worm, *Haemonchus contortus*, by the injection of a saline extract of the adult worms. The reader will find an extended and exceedingly interesting discussion of this subject in Culbertson (1941).

METHODS OF PROCEDURE

The methods used in caring for the experimental animals were the same as those employed in all the other experiments reported in this series of papers. The calves were secured when a few hours old and placed in individual cages with wire floors. The hay fed the animals was cut from land that had not been grazed by cattle for several years, and the cages were cleaned twice a week to insure that any hatched larvae were removed before they reached the infective stage. The calves were all obtained from the L. S. U. Dairy Department and were purebred Holsteins and Jerseys.

The stomach worm powder used in the injections was prepared from adult worms from calves killed at the Baton Rouge City Abattoir. They were washed 4 or 5 times in physiological saline solution, dried on filter paper at a tempera-



Figs. 1 to 4. Graphs of egg-counts of the four experiments described in the text.

ture of about 50° C. over night, and stored in cork stoppered bottles at room temperature until needed. The dried adult worms were prepared for injection by grinding the weighed amount in a sterilized mortar and suspended in 15 to 20 cc. of physiological saline. The injections were made with a sterile 10-cc. hypodermic syringe using a 16-gauge needle. The injections were made under the skin of the upper flank region of the calves.

The immune blood serum was prepared from freshly drawn blood that had been defibrinated by shaking in 50-cc. centrifuge tubes in which 3 or 4 glass beads had been placed. The tubes were then centrifuged until the erythrocytes were thrown down, and then the serum was withdrawn and stored in a serum bottle over night. The injections were made into the jugular vein from the serum bottle the following morning. All the equipment was sterilized before use and test cultures from the resulting serum indicated its freedom from bacterial infection.

EXPERIMENTAL DATA

Calf No. 142.—This animal was born on December 24, 1943. He was given infective larvae cultured from a calf with a pure infection of the large stomach worm on the following dates: May 24, 27, 30, and June 3, 1944. Equal amounts of the same suspensions of larvae were also given to calf No. 143 at the same time as a control. No. 142 was injected with powdered worm material as follows: 200 mg. on May 25, 210 mg. May 29, 180 June 1, 190 June 6, 210 June 10, 220 June 14, 160 June 17, 210 June 20, and 210 on June 23. It will be noted (Fig. 1) that the first eggs appeared in the manure of No. 142 on June 19 and No. 143 on June 20, 26 and 27 days respectively after the first inoculation. The maximum egg-count obtained from No. 142, the injected calf, was 2587 eggs per gram of sediment on July 16, and the maximum number recovered from the uninjected animal No. 143 was 778 on July 2. Daily egg-counts were made on both calves until the time they were killed, but, because of lack of space in the graph, only the maximum and minimum and some selected additional counts are shown. No. 142 was killed on July 17 and 9587 male and 8156 female stomach worms were recovered at the postmortem examination. No. 143 was killed on July 18 and 4447 male and 4974 female stomach worms were recovered. Thus it is evident from the comparative egg-counts and the number of worms recovered at the postmortem examination that the dried worm powder offered no protection against the large stomach worm in this experiment. No symtoms of parasitosis were observed in either calf.

Calf No. 146.—This animal was a purebred Jersey male born December 29, 1943. He was given a very large number of stomach worm larvae on the following dates: July 22, 24, and 28, 1944. An equal quantity of the same suspensions of larvae was given to calf No. 147 as a control on the same dates. No. 147 was also a purebred Jersey male and was born on December 30, 1943. No. 146 was injected with 110 mg. of worm powder on each of the following dates: July 24, 25, 26, 27, 28, 29, 31, and on August 2, 1944. The first eggs appeared in the manure of No. 146 on August 20 and in the manure of No. 157 on August 19 (Fig. 2). The egg-counts of both calves reached their maximum about August 30 and were essentially at the same level during the period of maximum egg production. The egg-counts fell rapidly during the next two weeks and by the middle of September were at a very low level. Because of lack of space only the first portion of the egg-count record is shown in Fig. 2. As judged by the relative number of eggs recovered from the manure the injection of the dried worm powder offered no protection against the large stomach worm when injected during the prepatent period. No symptoms of parasitosis were observed in either animal.

Calf No. 139.—This animal was a purebred Holstein male born March 2, 1943. On August 4, 1943 he was given a very small number of stomach worm larvae cultured from adults obtained from calves killed at the Baton Rouge City Abattoir. As a result of this inoculation he developed a relatively low egg-count between September 4 and November 9, only the later portion of which is shown in Figure 3 because of lack of space. The following amounts of stomach worm powder were injected in the right and left flanks on alternate dates: 230 mg. on September 10, 120 on the 22nd, 360 on the 25th, 260 on October 1, 200 on the 5th, and 320 on the 13th, 1943. On October 14, 21, and 22 he was given a very large number of infective larvae cultured from a calf with a mixed infection of the large stomach worm and the nodular worm. An equal amount of the same suspensions of larvae was given to calf No. 136 on the same dates as a control. No. 136 was a purebred Holstein male born January 28, 1943.

Both animals began to show distinct symptoms of parasitosis by November 5th. No. 136 developed a bad diarrhea on October 28, was unable to get up on November 7, and died on November 9. At the postmortem examination the fourth stomach was very much inflamed and a large number of mature and nearly mature large stomach worms were recovered from the contents. The posterior one-fourth of the small intestine and the anterior portion of the large intestine and caecum showed the usual inflammation associated with severe larval nodular worm infection. Very numerous larval nodular worms were recovered from the contents. A few stomach worm eggs were recovered from the manure of No. 136 on November 5 and 8 as indicated in Fig. 3. No. 139 began to develop a diarrhea as early as October 29 but did not begin to show marked evidence of loss of strength and appetite until November 13 when he began having difficulty in getting up. He also ate very little until his death on November 17. At the postmortem examination the fourth stomach was normal in appearance and 1145 mature and nearly mature large stomach worms were recovered from the contents. The posterior one-fourth of the small intestine and anterior portion of the large intestine were very much inflamed and a large number of immature nodular worms were recovered from the contents of each. It will be noted (Fig. 3) that there was a marked increase in the number of stomach worm eggs recovered from the manure of No. 139 following November 11, and that two positive fecal examinations were made on No. 136 just before death. It is evident from the above experiment that no protection was obtained from injections of stomach worm powder against inoculations with mixed cultures of stomach worm and nodular worm larvae.

Calf No. 118.—This animal was a purebred Jersey male born October 20, 1940. He was given the following amounts of blood serum prepared from calf No. 109 on the following dates: 145 cc. on January 15, 1941, 100 cc. on the 28th, 200 cc. on February 1, 230 on the 4th, 200 on the 11th, and 240 on the 22nd. On January 27 and 29, 1941 a large number of infective stomach worm larvae were administered in equal quantities to calves No. 118 and No. 120, No. 120 being used as a control. The details of the previous experiments with No. 109 are to be found in a previous report (Mayhew, 1945) and reference to Fig. 2 will show that he had been demonstrated to be immune by a rapid decrease in egg-count during August and his failure to become reinoculated as a result of the larvae administered the last part of August (Fig. 2 and text page 23). The resulting egg-counts (Fig. 4) indicated that the blood serum of a calf that had been demonstrated to be immune approximately 4 months previous offered no protection against infection with the stomach worm in a susceptible animal. Had it been possible to have carried out the experiment in early September instead of January such serum might be protective. It is also possible that larger quantities might be effective.

SUMMARY

Three experiments are described in which powdered large stomach worms (*Haemonchus contortus*) were injected subcutaneously in three non-immune calves in an attempt to develop an immunity to the large stomach worm. One experiment is described in which blood serum from a calf that had been demonstrated to be immune was given to a non-immune calf. The results indicate that no protection was offered by either the worm powder or blood serum.

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Some Observations on Cestodes of the Genus *Paranoplocephala* Luehe, Parasitic in North American Voles (*Microtus* spp.)[1]

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Although cestodes of the genus *Paranoplocephala* Luehe, 1910, are common parasites of voles and certain other rodents in North America, their taxonomic status to the present time has been much confused. It is the purpose of this paper to clarify the situation in regard to those species parasitic in voles of the genus *Microtus* Schrank.

While certain of the cestodes considered here occur in hosts of other microtine genera, they are by far more abundant in the various species of *Microtus*. At least two are also common in geomyid rodents. With the possible exception of *Paranoplocephala brevis* Kirschenblatt, 1938, discussed further below, none of the North American species has been recorded from Eurasian rodents.

The material upon which this work is based has resulted from the examination by one of us (R.R.) of nearly a thousand voles and related microtine rodents. Most of the animals were collected over the past six years from the North

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Central States region, although some were also collected in Wyoming and Manitoba. Five species of *Microtus* have been considered: *Microtus p. pennsylvanicus* Ord, *M. pennsylvanicus drummondii* (Audubon and Bachman), *M. pennsylvanicus modestus* (Baird), *M. montanus nanus* (Merriam), and *M. ochrogaster* (Wagner). The material resulting from the examination of hosts of other genera (*Pitymys, Synaptomys, Phenacomys, Clethrionomys,* etc.) is not considered here, since this is essentially an attempt to make it possible to differentiate the species parasitic in *Microtus.* It seems desirable to avoid any complications brought about by possible host variation.

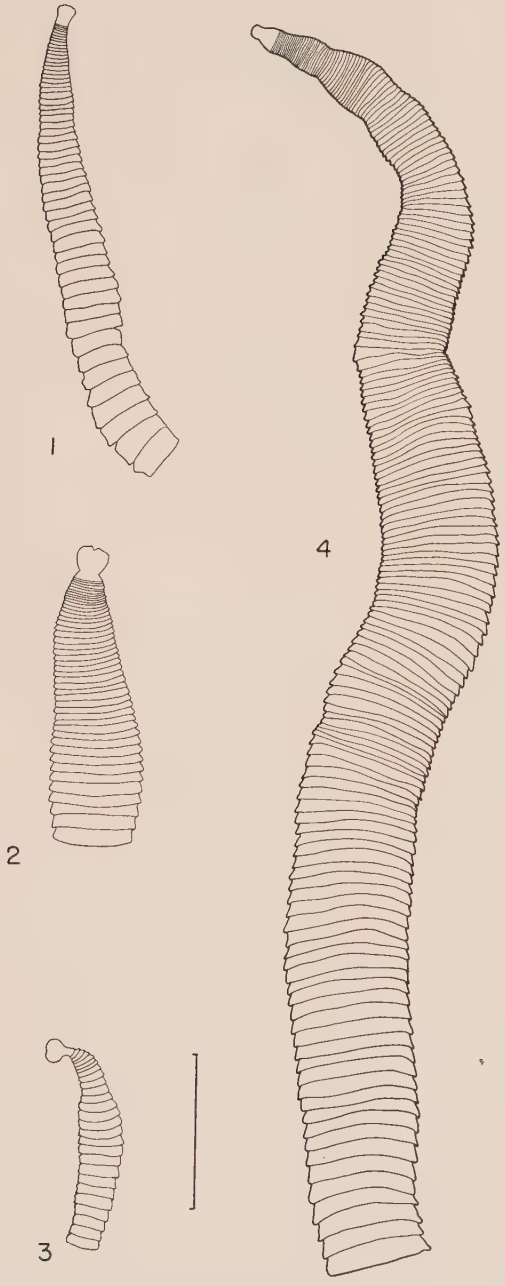
All material has been treated in the same manner, in order to avoid differences brought about by different degrees of relaxation of the cestodes. After being allowed to relax in tap water, the worms were killed and fixed, without pressure, by the application of hot formalin-acetic acid-alcohol solution. Whole mounts were routinely stained with Semichon's acetic carmine, and serial sections were stained with haematoxylin-eosin.

Early in our studies of the helminths of voles it was recognized that three distinct species were present, although it was not possible to identify them according to existing descriptions. Douthitt (1915), in his monograph of the cestode family Anoplocephalidae, described two species, *Anoplocephala variabilis* and *A. infrequens.* He further recognized a subspecies of the former, *A. variabilis borealis.* Douthitt's species were all described from *Geomys bursarius* (Shaw), although he also recorded *P. infrequens* from *Clethrionomys* (= *Evotomys*). Baer (1927) reassigned Douthitt's species to the genus *Paranoplocephala,* and, at the same time, indicated that *P. variabilis* was identical with *P. infrequens.* Rausch (1946) described *P. troeschi* from the common meadow vole. Only two species, therefore, have been considered as parasitic in North American rodents. Hansen (1947) considered *P. variabilis* and *P. infrequens* to be separate, on the basis of skirt-like extensions observed on specimens identified by him as *P. infrequens.* Harkema (1946) reported *P. omphalodes* (Hermann, 1783) from the cotton rat. Dr. Harkema kindly allowed us to examine his material, and it was found that his original identification was incorrect.

Since Douthitt's work relatively little has been done with these cestodes, except that they have been recorded from additional host species. Harkema (1936) and Erickson (1938) included *Paranoplocephala infrequens* in their lists of helminths from rodents. This species was also mentioned briefly by Rausch (1946) and by Hansen (1947). There has been a tendency by some workers to confuse the genera *Andrya* and *Paranoplocephala* when attempting to identify cestodes from voles. This is hardly justifiable in view of the form of the uterus in the two genera; other details, such as form of strobila, are characteristic for American species.

Through the kindness of Dr. E. W. Price we have examined 56 slides, mostly serial sections, of Douthitt's original material. Dr. Jean G. Baer has supplied slides of certain European forms, and Mr. Merle Hansen allowed us to examine his material from Nebraska voles.

After a study of Douthitt's material, we are of the opinion that *Paranoplocephala variabilis* should be considered a valid species. It was found that *P. infrequens* is a composite of what appears to be small specimens of *P. variabilis* and which has since been described by Rausch (1946) as *P. troeschi.* Examination of slides of the former led Baer (1927) to consider *P. variabilis* identical with *P. infrequens.* Since, when *P. troeschi* was described, the types of *P. infrequens* were not examined, and since there was no agreement with the diagnosis of the latter as given by Baer (1927), *P. troeschi* was not recognized as



FIGS. 1–4. Macroscopic appearance of species of *Paranoplocephala* parasitic in voles. The scale has the value of 5 mm. 1—*Paranoplocephala borealis* (Douthitt, 1915) n. comb. 2—*P. infrequens* (Douthitt, 1915). 3—*Paranoplocephala* sp. 4—*P. variabilis* (Douthitt, 1915).

being identical with it. The present study, however, has revealed that the name *P. troeschi* Rausch, 1946, must be considered a synonym of *P. infrequens* (Douthitt, 1915).

We are also of the opinion that Douthitt's subspecies, *Paranoplocephala variabilis borealis*, should be elevated to full specific rank, since it appears to be quite distinct.

This results, then, in three recognizable species; *Paranoplocephala variabilis* (Douthitt, 1915), *P. borealis* (Douthitt, 1915) n. comb., and *P. infrequens* (Douthitt, 1915). A fourth species, of uncertain status, is discussed below.

We have included a brief diagnosis for each species, based on material from voles. A key is also presented. It is ordinarily possible to recognize these species at the time they are removed from the host (see Figs. 1–4).

Paranoplocephala variabilis (Douthitt, 1915)

(Figs. 4, 8, and 12)

Synonyms.—*Anoplocephala variabilis* Douthitt, 1915; *Anoplocephaloides variabilis* (Douthitt, 1915) Baer, 1924; *Paranoplocephala infrequens* (Douthitt, 1915) in part.

Diagnosis.—Length 15 to 41 mm.; maximum width, attained in post-mature segments, 2 to 4 mm. Strobila much attenuated anteriorly, increasing gradually in width posterad. Segments from 42 to 199 in number, much wider than long (ratio of length to width about 1: 12 in mature segments), increasing somewhat in length in post-mature segments. Scolex from 210 to 370 μ long by 380 to 560 μ wide; relatively very small and weak. Suckers weakly developed, from 140 to 210 μ in diameter; neck short, of about same diameter as scolex. Muscle fibers strongly developed. Ventral and transverse longitudinal excretory canals measure from 10 to 20 μ in diameter. Dorsal excretory canals measure about 6 μ. Genital pores are unilateral, dextral, situated near middle of margin of segment. Genital ducts pass dorsal to longitudinal excretory canals. Testes, spherical, are 60 to 80 in number, and from 20 to 30 μ in diameter in mature segments. They extend from about middle of segment to beyond ventral excretory canal on aporal side; usually from 6 to 8 testes are found lateral to the ventral canal. Cirrus sac, elongate pyriform, measures from 115 to 200 μ long by 28 to 56 μ wide in mature segments. Cirrus aspinose. Cirrus sac does not reach across poral ventral excretory canal. Internal and external seminal vesicles well developed. Vagina opens posterior and partly ventral to cirrus sac. Seminal receptacle enlarges to extend from proximal end of cirrus sac to well beyond poral margin of ovary in mature segments. Ovary strongly lobed, situated somewhat porally near middle of proglottid. Vitelline gland, irregularly elongate, is partly posterior and dorsal to ovary. Uterus, tubular, extending ventrally beyond longitudinal excretory vessels; mostly ventral to ovary. Uterus becomes sacculate and finally fills entire gravid segment. Eggs measure from 20 to 26 μ wide by 26 to 33 μ long. Pyriform apparatus well developed.

A whole-mount of a typical specimen has been deposited in the Helminthological Collection of the U. S. National Museum, slide number 46371.

This is the largest species of the genus that we have found parasitic in voles (Fig. 4). It is commonly found in the duodenum of the host, with the scolex just below the pyloric valve. We have never observed more than one worm in a single host animal, although Douthitt (1915) reported as many as 50 specimens in a single *Geomys*. Douthitt's specimens were often larger, a situation perhaps brought about by occurrence in a different host species. This cestode is uncommon enough that we can make no statement concerning distribution or seasonal occurrence.

Paranoplocephala borealis (Douthitt, 1915) n. comb.

(Figs. 1, 5, and 9)

Synonym.—*Anoplocephala variabilis borealis* Douthitt, 1915.

Diagnosis.—Length 10 to 14 mm.; maximum width, attained usually near middle of strobila, 1.4 to 2 mm. Proglottids from 43 to 84 in number, much wider than long (ratio of length to width in mature segments about 1: 4); they increase somewhat in length as they become older, but are never longer than wide. Scolex from 284 to 355 μ wide by 230 to 270 μ long. Suckers from 100 to 130 μ in diameter. Scolex rather prominent, considerably wider than neck; suckers weakly developed. Muscle fibers not so strongly developed; living worm somewhat translucent. Ventral excretory canals 10 to 16 μ in diameter; transverse canals from 6 to 16 μ. Dorsal canals about 6 μ in diameter. Genital pores unilateral, sinistral, situated near middle of margin of proglottid. Genital ducts pass dorsal to longitudinal excretory canals. Testes ovoid, from 38 to 52 in number; from 20 to 30 μ in length in mature segments. Testes extend from aporal edge of vitelline gland to well across the aporal ventral excretory canal. They also extend anterior to the uterus and posterior to the transverse excretory canal in mature segments; this may be seen in segments that are well relaxed, so it cannot be attributed to state of contraction of the segments. From 5 to 12 testes may be found lateral to the aporal longitudinal excretory canals. Cirrus sac elongate, from 30 to 62 μ wide by 100 to 150 μ long in mature segments; it does not extend past ventral longitudinal excretory canal. Cirrus aspinose. Internal and external seminal vesicles well developed. Vagina opens posterior and somewhat ventral to cirrus sac. Seminal receptacle well developed, extending from proximal margin of poral ventral excretory canal to near middle of vitelline gland in mature segments; it becomes nearly spherical in older segments. Ovary, situated near middle of segment, strongly lobed; ovarian lobes relatively fine and elongate. Vitelline gland posterior and dorsal to ovary; rather semilunar in shape. Mehlis' gland spherical, just anterior to vitelline gland and situated in anterior depression of latter. Uterus tubular, becoming sacculate and filling entire segment when completely gravid. Eggs measure from 20 to 23 μ wide to 30 μ long. Pyriform apparatus well developed.

A whole-mount of a typical specimen has been deposited in the Helminthological Collection of the U. S. National Museum, slide number 46373.

We have observed no tendency in this species to intergrade with *Paranoplocephala variabilis,* although Douthitt was of the opinion that this occurred. In fact, we have collected both species in the same latitude in the North Central States region. It appears, however, that *P. borealis* is more common in the northern section of that region. On the basis of morphological differences, it seems entirely justifiable to consider *P. borealis* a distinct species. This cestode is found in the small intestine of the host.

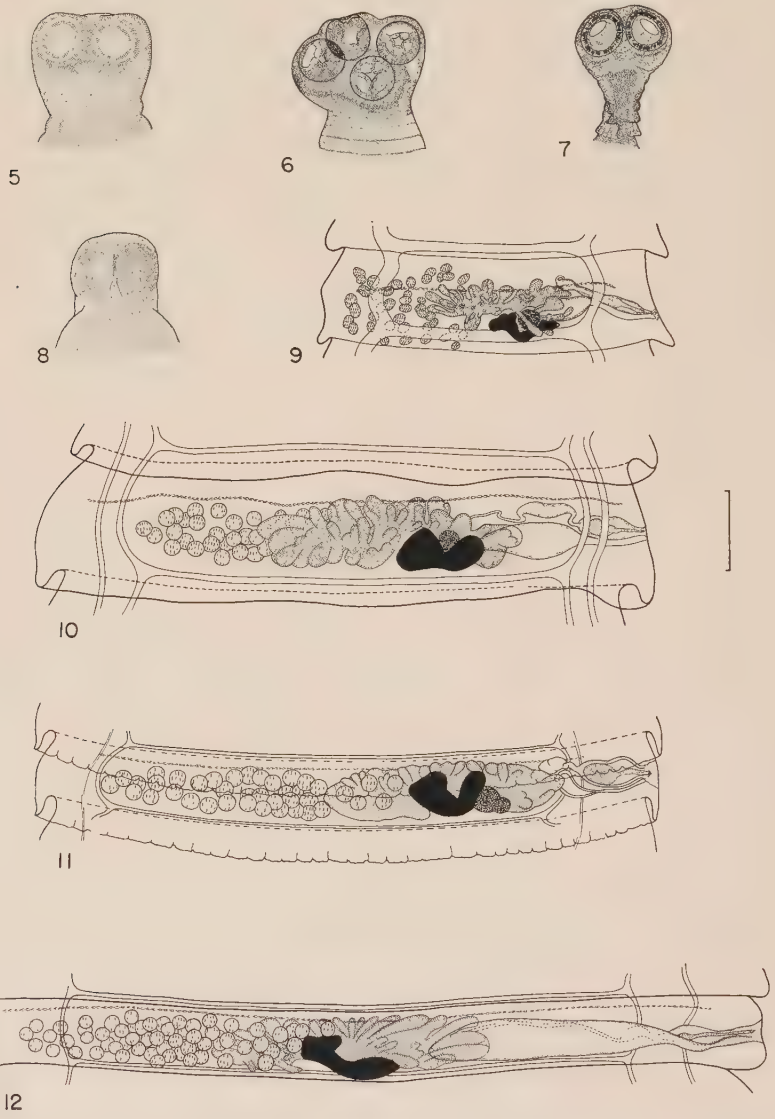
Paranoplocephala infrequens (Douthitt, 1915)

(Figs. 2, 6, and 10)

Synonyms.—*Anoplocephala infrequens* Douthitt, 1915; *Anoplocephaloides infrequens* (Douthitt, 1915) Baer, 1924; *Paranoplocephala variabilis* (Douthitt, 1915) in part; *P. troeschi* Rausch, 1946.

Diagnosis.—Length 4 to 11.5 mm.; maximum width, attained at posterior end of strobila, 1.3 to 3.5 mm. Strobila distinctly wedge-shaped; segments from 25 to 51 in number. Scolex from 640 to 940 μ wide by 568 to 710 μ long; very powerfully developed and quite distinct from the neck. Suckers, strongly developed, from 298 to 340 μ in diameter. Neck short and not prominent. Muscle

fibers strongly developed. Ventral excretory canals 14 to 22 μ in diameter; transverse canals 12 to 18 μ. Dorsal canals about 12 μ in diameter. Genital pores



Figs. 5–12. Morphological details of species of *Paranoplocephala* parasitic in voles. The scale has the value of 600 μ for figs. 5, 6, and 7; 900 μ for fig. 8; 200 μ for figs 9, 10, 11, 12. 5—Scolex of *Paranoplocephala borealis* (Douthitt, 1915) n. comb. 6—Scolex of *P. infrequens* (Douthitt, 1915). 7—Scolex of *Paranoplocephala* sp. 8.—Scolex of *P. variabilis* (Douthitt, 1915). 9—Mature segment, ventral view, of *P. borealis*. 10—Mature segment, ventral view, of *P. infrequens*. 11—Mature segment, dorsal view, of *Paranoplocephala* sp. 12—Mature segment, dorsal view, of *P. variabilis*.

unilateral, sinistral, situated at about middle of margin of segment. Genital canals dorsal to longitudinal excretory canals. Testes spherical, 35 to 50 in

number; from 33 to 50 μ in diameter. Testes extend from aporal edge of vitelline gland to aporal ventral longitudinal excretory canal, but never beyond it. Cirrus sac strongly developed, pyriform, from 30 to 66 μ wide by 100 to 155 μ long; it is often constricted near middle. Cirrus spinose. Internal and external seminal vesicles well developed. Vagina opens ventral and slightly posterior to cirrus sac. Seminal receptacle well developed, extending from proximal end of cirrus sac to vitelline gland in mature segments; it becomes much larger as segments become gravid. Vitelline gland, bilobed and somewhat reniform, situated dorsal to poral half of ovary. Mehlis' gland just anterior to vitelline gland, situated in anterior depression of the latter. Tubular uterus extends ventrally past longitudinal excretory canals; sacculations gradually develop, and uterus completely fills gravid segments. Eggs measure from 30 to 40 μ in diameter. Pyriform apparatus well developed.

A whole-mount of a typical specimen has been deposited in the Helminthological Collection of the U. S. National Museum, slide number 46372.

Paranoplocephala infrequens is the most common cestode occurring in voles in the central part of the United States. It was found in all vole species examined except for *Microtus pennsylvanicus drummondii* (collected in Manitoba).

TABLE 1.—*A comparison of the measurements of* Paranoplocephala brevis *Kirschenblatt, 1938, and* P. infrequens *(Douthitt, 1915).*

	P. brevis	*P. infrequens*
No. of segments	20 to 26	25 to 51
Length	5.6 to 6 mm.	4 to 11.5 mm.
Max. width	2.1 to 2.5 mm.	1.3 to 3.5 mm.
Diameter of scolex	830 to 860 μ	640 to 940 μ
Diameter of suckers	315 to 365 μ	298 to 340 μ
Genital pores	unilateral	unilateral
No. of testes	28 to 33	33 to 50
Length of cirrus sac	170 to 200 μ	100 to 155 μ
Diameter of eggs	26 to 38 μ	30 to 40 μ

The seasonal fluctuations of this species are of particular interest (Rausch and Tiner, 1948). *Paranoplocephala infrequens* is most commonly found in the cecum of the host, although at times it occurs just anterior to the cecum in the terminal portion of the small intestine. This species also occurs in larger numbers in a single host animal than do the two above-mentioned species.

Hansen (1947) indicated certain morphological details for this species (*"Paranoplocephala troeschi"*) which differed from those given by Rausch (1946). An examination of Hansen's material has revealed that he was in error as to the identification of part of his specimens, having included with those of *"P. troeschi"* measurements from another species.

Kirschenblatt (1938) described a cestode, *Paranoplocephala brevis,* from *Microtus* spp. from the Transcaucasus region of Russia. It is possible that *P. brevis* will be found to be identical with *P. infrequens,* since both morphologically and ecologically they are quite similar. Table 1 compares the measurements of the two species; certain differences may be found to disappear when a large series of *P. brevis* is studied. Further discussion of the ecology of the two species has been given in another paper (Rausch and Tiner, 1948).

Paranoplocephala sp.

Diagnosis.—Length from 6 to 8 mm.; maximum width, attained near the middle of the strobila, 1.5 to 2 mm. Anterior end of strobila more attenuated

than that of *P. infrequens*. Segments, from 30 to 32 in number, much wider than long (ratio of length to width of mature segments about 1: 9). Scolex from 582 to 710 μ wide by 454 to 525 μ long. Suckers from 284 to 326 μ in diameter. Scolex distinct from neck, but suckers not so prominently developed. Neck well defined, about $\frac{2}{3}$ as long as scolex. Musculature rather weakly developed. Ventral longitudinal excretory canals from 12 to 23 μ in diameter; transverse canals of a similar size. Dorsal canals from 4 to 7 μ in diameter. Genital pores unilateral, dextral, situated near middle of margin of segment. Genital ducts pass dorsal to longitudinal excretory canals. Testes spherical, from 40 to 50 in number; from 20 to 33 μ in diameter in mature segments. Testes extend from aporal edge of vitelline gland to aporal ventral longitudinal excretory canal, but not beyond it. Cirrus sac pyriform, from 156 to 185 μ long by 57 to 71 μ wide. Internal and external seminal vesicles well developed. Vagina opens posterior to cirrus sac. Seminal receptacle well developed. Ovary more sacculate than lobate, situated usually in poral half of segment. Vitelline gland dorsal to middle of ovary; reniform in shape. Mehlis' gland poral to vitelline gland. Tubular uterus extends ventrally past longitudinal excretory canals. Uterus becomes sacculate and finally fills entire gravid segment. Eggs measure from 26 to 33 μ wide by 33 to 40 μ long. Pyriform apparatus well developed.

A whole-mount of a specimen has been deposited in the Helminthological Collection of the U. S. National Museum, slide number 46374.

Although superficially this species closely resembles *Paranoplocephala infrequens*, certain differences make it doubtful that they are identical. The scolex of the present species is not so strongly developed, although Baer (personal communication) does not consider this character to be of value in this genus. In well-relaxed strobilae the skirt on the posterior margin of the segments extends much farther posterad than that of *P. infrequens*. The ovary is more poral, and the vitelline gland is at the center of the ovary, instead of above the poral half. Moreover, the genital pore in this species is dextral, rather than sinistral as it is in *P. infrequens*. We can not say whether this character is subject to such variation, but the study of material available so far would indicate that it is not. As with the foregoing species, this cestode is found in the cecum of the host. Until further material is available for study it seems best to leave the status of this worm indefinite.

Key to the species of Paranoplocephala parasitic in North American voles.

1. Testes extend beyond aporal ventral longitudinal excretory canal 2
   Testes do not extend beyond aporal canal ..... *P. infrequens.*
2. Testes from 60 to 80 in number; size large; ratio of length to width of mature segments about 1: 12 ..... *P. variabilis.*
   Testes from 38 to 50 in number; size small; ratio of length to width of mature segments about 1: 4 ..... *P. borealis.*

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A Method of Staining *Trichomonas foetus* in Smears of Bovine Vaginal Secretions

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The difficulties encountered in making satisfactory stained preparations of *Trichomonas foetus* have been pointed out by various authors. Applications of standard protozoological methods have, in most instances, produced unsatisfactory results owing to loss or distortion of the organisms during smearing, fixing, and/or staining.

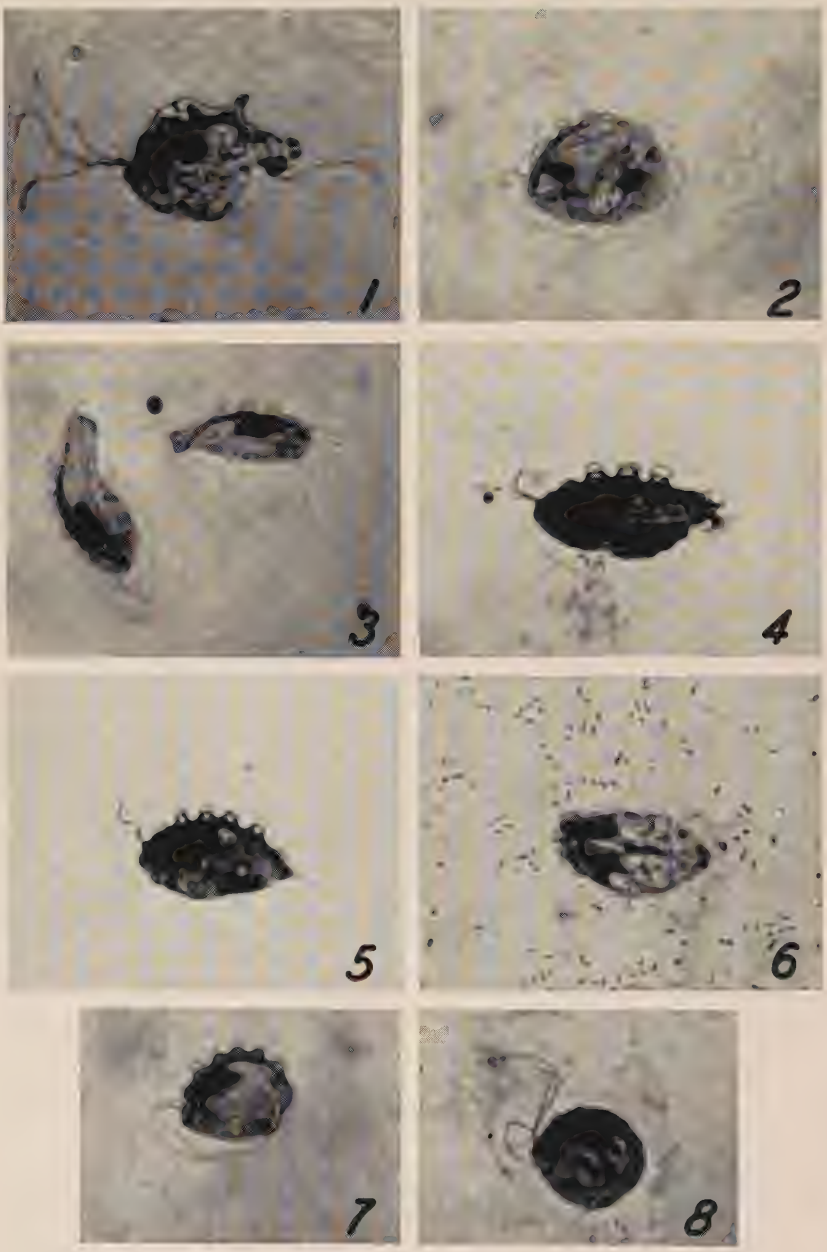
Van Someren (1946) reported failure with standard methods employing wet fixation in Carnoy's, Schaudinn's, and Bouin's fixatives, or vapor fixation with formalin and similar reagents. He found that Kent's method of wet fixation in iodine solution, followed by staining in Giemsa's stain, Delafield's hematoxylin, or Heidenhain's iron hematoxylin, gave unusually good results. Stableforth and Scorgie (1937), employing dry or moist treatment with osmic acid, Bouin's fixative, or methyl alcohol followed by Giemsa's stain or Heidenhain's iron hematoxylin stain, obtained only a few satisfactory preparations in which but few specimens were suitable for study. In their morphological studies of *T. foetus,* Wenrich and Emmerson (1933) used vaginal smears fixed in Schaudinn's, Hollande's, or chrom-acetic fixatives, and stained with Giemsa's stain (wet method) or iron-alum hematoxylin.

Cameron (1935, 1938) reported fair results with wet fixation in Schaudinn's solution, followed by Giemsa's stain. He emphasized the importance of keeping the organisms moist during fixation. Gehring and Murray (1933), using the Romanowsky-Giemsa blood smear technique, produced satisfactory preparations from air-dried vaginal smears. These authors noted that better smears resulted from a watery, mucous discharge than from uterine pus or heavy vaginal mucus. The photomicrographs accompanying their publication are excellent. Stewart (1938) reported good results with a rapid method (15 min.) involving osmic acid fixation followed by Wright's blood stain.

It is surprising that workers who obtained occasional good results with standard methods did not attempt to determine the factors responsible for their occasional successes instead of condemning and discarding these methods. To the writers' knowledge, no such investigations have been made, the frequency of failures prompting only the search for new procedures especially designed for staining *T. foetus.*

In connection with the bovine venereal trichomoniasis investigations con-

[1] Resigned January, 1947.
[2] Resigned January, 1948.



FIGS. 1–8. Unretouched photomicrographs of *Trichomonas foetus*, ×1600. All specimens were fixed in Bouin's fixative and stained with Heidenhain's iron hematoxylin except fig. 6 which was stained in Harris' hematoxylin and counterstained with eosin. 1—Large form displaying bulbous posterior region. 2—Form showing unusual relationship of structures; possibly a division stage. 3—Two specimens with body shape and structure most typical of *T. foetus*. 4—Specimen in which the endoaxostylar granules in the capitulum of the axostyle are well defined. 5—Specimen showing tip of axostyle. 6—Division stage showing two sets of structures. 7—Rounded form displaying conformation of the axostyle to body shape and an unusually long posterior flagellum. 8—Rounded form exhibiting endoaxostylar granules in the capitulum of the axostyle. Photographs by M. L. Foubert, Photographer (retired), U. S. Department of Agriculture.

ducted in the Zoological Division, stained preparations of *T. foetus* from infected cattle were frequently required. However, the limited time available for this work precluded the employment of special procedures and precautions in preparing the slides. Routinely, therefore, thin, moist smears of vaginal exudate were placed in Bouin's fixative, washed in tap water, mordanted in 4 per cent iron-alum, stained with Heidenhain's iron hematoxylin, differentiated in 2 per cent iron-alum while viewed under the 4 mm. (high-dry) objective of the microscope, dehydrated, cleared, and mounted in clarite-X. No special consideration was given the time of fixation, mordanting, etc. Although most of these slides were unsatisfactory, a few were excellent (Fig. 1).

These results were in accord with those reported by other authors and demonstrated that acceptable slides of *T. foetus* could be prepared by the methods employed. Consequently a series of 34 slides was prepared in an effort to determine precisely the factors responsible for occasional good results. The method of preparing the smears, the degree to which they were allowed to dry before fixation, and the time of exposure of the smears to the various reagents were varied in an effort to ascertain which procedures would consistently produce optimum results.

The resulting preparations were rated as excellent, satisfactory, and unsatisfactory. Of the 34 slides prepared, 5 were rated excellent, 6 satisfactory, and 23 unsatisfactory. Conditions for the rating of satisfactory were (1) that the preparation contain large numbers of undistorted *T. foetus* evenly distributed throughout the smear, and (2) that the anterior flagella, posterior flagellum, undulating membrane, axostyle, nucleus, blepharoplast, and costa be clearly discernible and demonstrable (Figs. 3 and 7). Preparations to be considered excellent should show, in addition to the above mentioned features, certain structures which are not frequently seen in stained preparations of *T. foetus*, namely, the endoaxostylar granules in the capitulum of the axostyle, terminal spine of the axostyle, and the chromatic ring about the axostyle at the point of emergence from the body (Figs. 1, 4, 5, 7, and 8). Preparations which did not meet these requirements were considered unsatisfactory.

Examination of the procedures which resulted in the production of satisfactory and unsatisfactory preparations shows that (1) smears should be made from a small quantity of mucus spread evenly and thinly with a wire loop, smears made by spreading the mucus with the edge of a coverslip being completely unsatisfactory; (2) wet fixation[3] in Bouin's fixative gave best results provided the smears were very thin. Moist fixation[4] was unsatisfactory because of the difficulty of judging the extent of the dryness of the mucus and the consequent loss of many organisms by desiccation; (3) all periods of fixation from 5 minutes to 27 days were equally satisfactory; (4) mordanting time appeared to be the most critical factor governing results, mordanting times of 3 hours, 3½ hours and 9 days resulting in a greater proportion of satisfactory preparations than mordanting times of 1 hour and 12 days.

The results obtained with the 34 slides previously mentioned suggested the following method of procedure for obtaining satisfactory slides:

1. Make thin, even smears from small quantities of vaginal secretions containing large numbers of *T. foetus*.
2. Fix wet smear in Bouin's fixative for 1 hour.
3. Wash in tap water for 5 minutes.

[3] In "wet fixation" smears were placed in fixative immediately after being made.

[4] In "moist fixation" smears were permitted to dry partially before being placed in fixative.

4. Mordant in 4 per cent iron-alum for 24 hours.
5. Wash in tap water for 5 minutes.
6. Stain in Heidenhain's iron hematoxylin for 2 hours, or until organisms are overstained.
7. Wash in tap water for 5 minutes.
8. Flood slide with 2 per cent iron-alum and observe destaining under 4 mm. objective of microscope.
9. Wash well in several changes of tap water.
10. Dehydrate in 95 per cent alcohol and absolute alcohol—5 minutes in each.
11. Clear in xyol for 5 minutes.
12. Mount in clarite-X or balsam.

This method of procedure was tested on two series of smears made from the vaginal mucus of an infected cow, 48 and 24 hours, respectively, prior to estrum. Each series consisted of 10 smears. The first series was made from thin, vaginal mucus obtained 48 hours prior to estrum and containing 220,000 *T. foetus* per cc.; the second series from the slightly thicker vaginal mucus obtained from the same cow 24 hours prior to estrum and containing 800,000 *T. foetus* per cc.

All the smears of the first series were unsatisfactory because they did not contain sufficient numbers of organisms to make routine detection and study practicable. Individual specimens of *T. foetus,* however, were morphologically normal and well stained (Figs. 4 and 5). Eight smears of the second series were considered satisfactory and two were rated excellent. Undistorted, well stained specimens of *T. foetus* were numerous and their morphological characters sharply defined.

The two series differed widely in numbers of organisms on the slides, although the slides were uniform within their respective series. The relatively lower numbers of *T. foetus* in the material used for preparing the first series (220,000 per cc.) than in that for the second series (800,000 per cc.) does not logically account for the differences which resulted, as some smears prepared from mucus having numbers of organism lower than the former produced satisfactory slides in the previously mentioned series of 34 smears. Recognized differences in consistency of the mucus suggest a more probable explanation, although a specific property responsible for the loss of organisms was not determined. The answer might be found by preparation of smears with material collected throughout the course of an infection (through several estrual cycles) and correlating the characteristics of the mucus with the quality of the slides produced. Nevertheless, the results of these studies, as shown by the accompanying photographs, were considered adequate evidence that satisfactorily stained smears of *T. foetus* can be produced in quantity by the method described here.

DISCUSSION AND CONCLUSIONS

As may be seen from the observations reported here, the iron-hematoxylin method of staining Protozoa may be satisfactorily adapted to the staining of *T. foetus* in bovine vaginal secretions. However, for ease in observing destaining, as well as for profitable study of completed smears, the presence on the slides of large numbers of *T. foetus* is necessary. The procedure, incorporating no special reagents or tedious manipulations, is especially adapted to the routine preparation of slides.

Preparation of stained smears cannot be recommended as a standard diagnostic procedure for bovine venereal trichomoniasis as, ordinarily, trichomonads when present are more readily recognized in fresh material under low power of a microscope. However, stained specimens can be of value when it is necessary

to establish definitely the identity of suspect organisms by comparison with descriptions (such as that of Wenrich and Emmerson, 1933) of the morphology of *T. foetus*.

Should the technique described here be utilized as an aid in herd diagnosis, samples should be examined only from properly selected females at times when significant trichomonad populations can logically be expected to be present in the vagina (cf. Bartlett and Hammond, 1945). The populations of *T. foetus* in the prepuce of infected bulls are always relatively low; therefore, making stained preparations from preputial samples is not recommended.

SUMMARY

1. The literature revealed that irregular, though sometimes satisfactory, results were obtained after staining *T. foetus* by standard protozoological methods.

2. Our previous attempts to stain *T. foetus* by the protozoological methods in common use gave erratic results. Since no effort had been made to determine the factors responsible for occasional good results, the determination of these factors was considered worthwhile.

3. Preliminary studies were conducted to standardize a procedure which would yield a good proportion of well stained smears. This procedure was subsequently tested and satisfactory results were obtained in one of two test series. Unsatisfactory results in the other test series could be attributed either to the nature of the mucus or to the scarcity of organisms in it.

4. The preparation of stained smears cannot be recommended as a routine method of diagnosis of bovine venereal trichomoniasis, but may be of value under certain conditions.

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An Instance of Duplication of the Cement Glands in an Acanthocephalan

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In an article now in press (Van Cleave, Jour. Morphol.), attention has been directed to the fact that cement glands in males of the Acanthocephala furnish relatively stable characters at all taxonomic levels. Within the higher categories the number of glands is relatively less significant than the fundamental structure of each gland. In some species, what seems to be normal individual variability

in number has been observed by various writers. Thus in the orders Archiacanthocephala and Palaeacanthocephala specimens are occasionally encountered with reduced number of cement glands. These cases may either represent normal tendency to vary or effects of injury. Similarly, on rare occasions, a relatively small increase in number of cement glands has been recorded. At times this is attributable to faulty observation, due to crowded condition of the glands, but in some instances actual increases in number have been seen. Such was the case when Machado-Filho (1946) demonstrated ten cement glands for a specimen of Moniliformis where eight are the normal complement.

In a study involving examination of several hundred males of *Echinorhynchus gadi* Mueller from marine fishes of broad geographical dispersal, one instance of a very unusual abnormality was encountered. In this specimen (Fig. 1),

FIG. 1. Posterior extremity of a male of *Echinorhynchus gadi* from intestine of *Urophycis tenuis*, Isles of Shoals, New Hampshire, showing distribution of the twelve cement glands (C G) in their relations to the testes (T).

there is complete duplication of the cement glands so that twelve glands take the place of the six customary for the species. Duplication of this sort is probably the result of some disturbance during morphogenesis. Hamann (1891) and others have observed that in the Palaeacanthocephala the earliest primordium of each cement gland is a single embryonic cell. In normal development, the embryonic nuclei have lost their power of reproduction by mitosis before the rudiments of the somatic organs are laid down. This instance of complete duplication of the cement glands in *E. gadi* seems to be attributable to the operation of some factor leading to one additional division of the primordia before differentiation of the glands started.

The occurrence of the abnormal specimen in a large population of normal individuals and complete agreement between it and other males in the same host and the same locality in all other morphological details, eliminates the possibility of regarding this specimen as representing a distinct species.

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ERRATUM

Daniel S. Jaquette has called the Editor's attention to an error in his paper on *Trichomonas gallinae* infections in pigeons which appeared in Vol. 15, No. 2 of these Proceedings.

Bottom of Page 72 and continuing on page 73. The sentence beginning "Twenty-nine naturally infected birds—" should read as follows: "Twenty-nine naturally infected birds were kept under observation for periods ranging from 228 to 744 days and at the end of this period all but one (cock number 1212) were still infected."

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